REMARKS

Restriction

In response to Applicants' election of claims 1-12 and 25, with traverse, the Examiner indicates that there is no allowable generic or linking claim and therefore, no basis on which to rejoin the claims.

In response, Applicants re-emphasize that claims 12-24 should be rejoined in the instance that claims 1 or 11 are determined to be allowable in response to the Remarks presented herein.

Put simply, Applicants assert that claims 12-24 are not directed to a separate and distinct invention since they recite a use for the assay system recited in claim 11. Accordingly, any correctly designed prior art search for a transformed cell comprising the recited elements would necessarily uncover references that described using such a transformed cell, and thus, would not present an undue burden to the Examiner.

Accordingly, Remarks made herein are in support of patentability of claims 1-25, inclusive.

Rejection Under 35 U.S.C. §112-Indefiniteness

Claim 11 stands rejected as allegedly indefinite for lacking recitation of a specific number of transformed cells that will be used in the assay in order to determine if a test compound increases or decreases expression of a reporter gene contained within the cells. The Examiner alleges that the term "sufficient", referring to the number of transformed cells used, does not clearly define the metes and bounds of the claim.

This rejection is respectfully traversed. Applicants assert that the term "sufficient", when referring to the number of cells used in assays employing reporter genes for qualitatively and quantitatively determining levels of transcription, is definite in the pertinent art.

According to the MPEP §2173, a claim is indefinite when it fails to inform the public of the boundaries of what constitutes infringement of the claim. Secondarily, the definiteness requirement is to provide a measure of what the Applicants regard as their invention in order to permit a determination of enablement during prosecution.

A reporter gene, when operatively linked to coding and/or regulatory gene sequences of interest in a cell, will be co-expressed at the same level as the coding and/or regulatory sequences of interest. As such, expression levels of the sequences in response to agents that may increase or decrease expression can be quantitatively assessed by measuring reporter gene expression. However, expression of both the reporter gene and the gene/sequences of interest will depend on additional factors such as cell type, and the "strength" or "potentness" of the regulatory elements (i.e., promoter and/or enhancer) that are operatively linked to (or associated with), and drive transcription of, the reporter gene/sequences of interest. Stated another way, the same promoter/enhancer sequences will not result in uniform expression levels of their associated sequences in different cell types. This is demonstrated by the enclosed abstract by Nunez et al., *Mol Cell Endocrinol.* 2002; 189: 191-99 (Exhibit A). Nunez et al. demonstrate that three promoters which regulate expression of the human glucocorticoid receptor are equally effective at driving luciferese gene (the reporter gene) expression in human cervical carcinoma cells (HeLa), while in T-cell lymphoma cells (Jurkat), two of the three are equally effective and one is less effective. In yet another cell type, liver carcinoma cells (HepG2), the less effective promoter in Jurkat cells, is the most effective amongst the three.

The preceding therefore demonstrates that one of ordinary skill in the art appreciates that the absolute number of cells used in a reporter gene assay, such as the claimed assay, will depend on how efficiently the reporter gene (and, hence, sequences of interest) is expressed in the particular cell of interest, as driven by its promoter and/or enhancer. Accordingly, the optimal number of transformed cells will necessarily be experimentally determined for each cell type transformed to express the reporter gene construct. As an example, the present inventors demonstrate that 35 µl of a 200 µl cell suspension of 1×10^7

HepG2 cells (a little less than1/6), or about 1,750,000 cells, is a sufficient number of cells (see page 21 of specification, first and second paragraphs) for use in the claimed assay. However, this does not necessarily mean that this will be a sufficient number of cells of another type, or that fewer cells will not be sufficient if used in another cell type.

Furthermore, the number of cells required in the assay system depends on the nature of the reporter gene, which simply needs to be detected. The sensitivity of a light-emitting reporter, (e.g., luciferase) may differ from a chromogen-forming reporter gene product, like chloramphenicol acetyl transferase (CAT), or a protein that is detected immunologically, such as with a FLAG tag.

It is further asserted that the present claim language would pose no question as to whether a party was infringing the claim, as set forth in MPEP §2173, referenced above. Any person, in attempting to measure transcriptional activity of HL via reporter gene expression in a cell expressing the elements of transformed cell of the present claims, would infringe claim 11 at the instant expression of the reporter gene was observed. Similarly, the present claim language permits a determination of enablement for the same reason; the number of transformed cells is sufficient when the amount is such that reporter gene expression is observed. Such cell titration is routinely performed by those skilled in the relevant art, and is not considered to be "undue experimentation".

In view of the foregoing, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. §103(a)-Obviousness

Claims 1-11 and 25 stand rejected as obvious. According to the Examiner, the combination of Ameis et al. and Harnish et al., in view of secondary references Norris et al., Cullinan et al., Kumar et al., or Dicheck et al., teach a transformed cell expressing (i) a functional estrogen receptor (ER); (ii) a C/EBP transcription factor, and (iii) a reporter gene operatively associated with an HL promoter, and a method of using the cell in screening assays to find HL modulators.

This rejection is respectfully traversed. To meet the burden of *prima facie* obviousness under 35 U.S.C. §103(a), the Examiner must establish that three criteria have been met. First, there must be a concrete suggestion or motivation to modify what is taught in a reference or to combine its teachings with other references. Second, there must have been a reasonable expectation that the modifications or combination would succeed. Finally, the combined or modified prior art must actually teach all of the claimed limitations. Both the motivation and the reasonable expectation of success must be found in the prior art and not in Applicants' disclosure. See, M.P.E.P §2143; citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The present claims are not obvious because one of ordinary skill in the art would not have been motivated to combine the teachings of the aforementioned references to arrive at the invention defined by such claims. This is detailed further below.

Harnish discloses that estrogen regulates expression of apolipoprotein AI (ApoAI), via regulation of the ApoAI promoter sequences, along with additional co-activators. Such co-activators are listed on page 9270 of the reference (second column, bottom), and include p300 and CBP. Of note, CBP is not the C/EBP of the present claims. CBP is an acronym for **C**REB-**b**inding **p**rotein (CREB is, in turn, an acronym for **c**AMP **r**esponse **e**lement **b**inding protein). By contrast, C/EBP of the present invention refers to **C**CAAT enhancer **b**inding **p**rotein, an altogether different protein than CBP. Harnish also discloses an assay in which HepG2 cells are transfected with a construct encoding ERα, a construct containing the ApoAI basal promoter associated with a reporter gene, and a construct encoding the HNF-4 ApoAI-specific transcription factor.

Ameis discloses the isolation and characterization of the human HL gene, including that it has two CCAAT elements at -469 and -1228 upstream of the translation initiation site. Other *cis*-acting elements are also disclosed. Ameis does not disclose that HL is regulated by ER.

One of ordinary skill in the art would not have been motivated to combine the teachings of Harnish and Ameis for the following reasons. First, there is no teaching or suggestion in either reference that HL, like ApoAI, is regulated by ER, and hence, no motivation for one of ordinary skill in the art to combine the teachings and transfect an ER-containing construct, along with the promoter region of a completely distinct gene (i.e., HL).

The same applies for the construct comprising C/EBP. While Ameis discloses that there are two CCAAT elements that are characteristic of eukaryotic promoters, as indicated above, other *cis*-acting elements are also disclosed, including sequences that bind glucocorticoid receptor and cAMP, and undefined "Alu" repeat sequence. C/EBP is not specifically disclosed. Accordingly, one would not be motivated to select only the CCAAT-binding C/EBP transcription factor to co-transfect with the HL promoter, with any reasonable expectation of success that the specific combination would result in a high level of reporter gene expression driven by the HL promoter. While it may have been obvious to *try* co-transfecting each putative *cis*-acting regulatory element, or combinations thereof, along with the HL promoter until maximum transcription was achieved, "obvious to try" is not the standard for *prima facie* obviousness under 35 U.S.C. § 103. The Examiner's attention is directed to the Federal Circuit's decision in *In re O'Farrell*, 853 F.2d 984, 7 USPQ2d 1673 (Fed. Cir. 1988). In particular, the court notes:

> In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.

In fact, Applicants assert that one of ordinary skill in the art would have been more apt to select transcription factors that bind to the sequence AGGTTAATTATTAAT for co-transfection with the HL promtoer, over the more general CCAAT sequence-binding C/EBP

(which itself, is not even disclosed in Ameis) and expect a reasonable measure of success, since this sequence binds hepatocyte-specific factors and is present in several liver genes (Ameis).

In summary, neither Harnish nor Ameis provide the motivation to combine their respective teachings, and the Examiner is incorrectly relying on the teachings of the instant specification to provide such motivation. In addition, even if improperly combined, the teachings of Harnish and Ameis do not teach every claim limitation, since there is no direction or guidance provided by either reference that ER regulates the HL gene, or that general transcription factor C/EBP will drive expression of the HL promoter and the associated reporter gene, much less that it would do so in connection with ER. Since the improperly combined references fail to teach the claimed invention, there can be no reasonable expectation of success.

The secondary references do not supply the missing motivation to combine the references. Norris teaches a new subclass of DNA "Alu" repeat sequences that function as enhancers for ER regulated genes. Norris further teaches that this sub-class of "Alu" sequences have been identified within the promoter of HL, a "suspected target" of estrogen action (see page 22781, last sentence). Norris also describes cloning "individual Alu consensus sequences" into a vector in an attempt to confer ER responsiveness to a heterologous promoter in HepG2 cells in the presence of ER.

While Norris provides the teaching that HL *may* be regulated by ER due to the presence of "Alu" enhancer sequences, the suggestion in Norris does not meet the second requirement for obviousness described above, alone or in combination with Harnish and Ameis. Namely, the combined teachings do not provide a reasonable expectation that transformed cell expressing only the HL promoter, ER, and a general transcription factor (that binds to a general consensus sequence, CCAAT) will elicit a detectable level of reporter gene transcription. If anything, Norris, in combination with Harnish and Ameis, would have motivated one of ordinary skill in the art to introduce additional "Alu" sequences into the cell in combination with the HL promoter and ER in order to elicit reporter gene activity. None of Harnish, Ameis or Norris teach or suggest that C/EBP is a critical transcription factor driving activation of the HL

promoter, so this combination of references cannot teach or suggest the subject matter of the present claims.

Cullinan et al. (U.S. Patent 5,908,859-"the '859 patent"), teaches small molecule compounds that down-regulate expression of HL, thereby increasing HDL levels, which can be used as therapeutics for hypercholesteremia. The '859 patent discloses, at column 2, lines 32-53, that the presence of estrogen also down-regulates the HL gene and may be responsible for the rise of HDL cholesterol. The '859 patent does not disclose or suggest transforming a cell with ER, the HL promoter, and C/EBP to screen for compounds that modulate HL activity via the ER. The '859 patent also does not disclose that C/EBP regulates HL in any way. Instead, the '859 patent discloses feeding the claimed compounds to *non-estrogen containing* rats (males or ovariectomized females) to determine their effect on plasma lipid levels.

Similar to Norris, while the '859 patent teaches that ER negatively regulates the HL gene, the '859 patent does not provide the motivation to engineer a transformed cell that can be used to identify compounds that regulate HL *via the ER*. To the contrary, the '859 patent teaches away from the present claims by administering compounds that directly inhibit endogenous liver HL, in animals lacking estrogen (i.e., in animals who are unresponsive to estrogen). Since the '859 patent teaches away from ER-regulated HL expression, there would have been no motivation to combine the '859 patent with Harnish or Ameis or both and arrive at the presently claimed invention. Harnish teaches that ER regulates the ApoAI promoter, and hence, his assay *relies* on the addition of estrogen to function. Ameis teaches only the human HL coding and putative regulatory sequences, which are distinct from endogenous rat HL disclosed in the '859 patent. As indicated above, the '859 patent does not disclose transforming any cell. Thus, there would be no motivation to combine the teachings of these references.

Moreover, the combination of Harnish, Ameis and the '859 patent, even assuming *arguendo*, motivation to do so, would not teach the limitations of the present claims, since the patent does not disclose transforming *any* cells with any constructs, does not disclose that ER

regulates HL via its promoter, and certainly does not disclose that C/EBP is required for enhanced activation of the HL promoter.

Kumar discloses functional domains of the human ER that are responsible for hormone binding and nuclear binding. Kumar describes that deletion of the N-terminal domain of the ER does not affect the ability of ER to activate a reporter gene containing a vitellogenin estrogen-responsive promoter but it impairs activation of the human pS2 gene promoter, as determined using reporter genes. Absent any other teaching, Kumar adds nothing to the teachings of Harmish and Ameis that would motivate one of ordinary skill in the art to engineer a transformed cell of the present claims, and reasonably expect that it would enable activation of a reporter gene in the presence of ER. The present claims require a construct containing "a functional estrogen receptor", in combination with the HL promoter and a C/EBP transcription factor. Since neither Harmish nor Ameis disclose that ER regulates HL, there would be no motivation for one of ordinary skill in the art to utilize the ER or any region thereof in an engineered cell designed to express HL, much less to do so in addition to C/EBP.

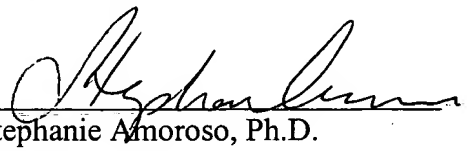
Lastly, Dichek discloses that overexpression of human HL in transgenic mice decreases levels of ApoB and HDL-containing lipoproteins. The constructs disclosed by Dicheck include human HL *coding sequences*, and promoters from the ApoE liver specific gene (see page 1897, column 1, Materials and Methods). Accordingly, Applicants do not see how Dichek would render obvious claims directed to a transformed cell containing an HL promoter, since Dichek teaches a construct lacking the HL promoter, but containing only the HL coding sequences driven by another promoter. In addition, absent the HL promoter, the combination of Harnish, Ameis and Dichek would have provided no motivation to co-transform a construct comprising ER, the HL promoter, and general transcription factor C/EBP, since none of the three references disclose ER or that HL is regulated by ER, and since none of the references disclose that C/EBP is required for efficient activation of the HL promoter.

In view of the above, it is respectfully requested that the obviousness rejection be withdrawn.

Therefore, in view of the above remarks, it is respectfully requested that the application be reconsidered and that *all* pending claims be allowed and the case passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



Stephanie Amoroso, Ph.D.
Reg. No. 51,401
Agent for Applicants

DARBY & DARBY, P.C.
Post Office Box 5257
New York, NY 10150-5257
Phone (212) 527-7700